

# HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF THE ANGIOGENESIS IN CANINE GLIOMA

## INTRODUCTION

Neovascularization has been described in gliomas through five different mechanisms<sup>1</sup> (Fig.1). However, Tumour growth and metastasis in animals depends basically on Angiogenesis<sup>2</sup>. **Vascular endothelial growth factor (VEGF)** is the most important driver of vascular formation and **vascular endothelial growth factor receptor 2 (VEGFR-2)** mediate the major growth and permeability actions of **VEGF**<sup>3</sup>. Cell adhesion protein **CD31** is often used as an indirect measure of angiogenesis both in canine and humans.

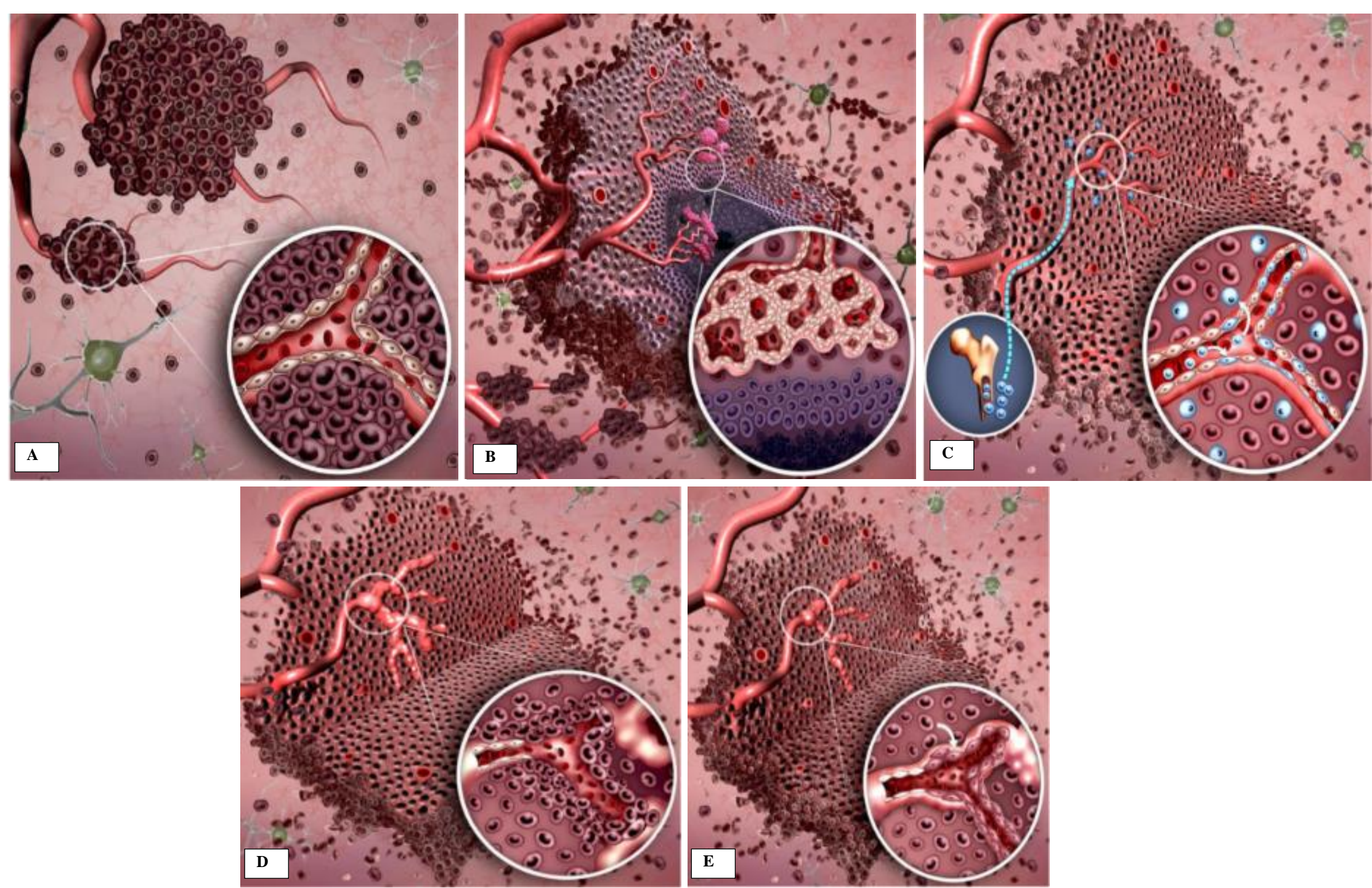


Fig. 1. Different mechanism by which glioma achieves neovascularization. (A) Vascular co-option (B) Angiogenesis (C) Vasculogenesis (D) Vascular mimicry: (E) Glioblastoma endothelial cell transdifferentiation.

## OBJECTIVE

The aim of this study was to identify and evaluate the neovascularization and angiogenesis processes through the histological and Immunohistochemical analysis of spontaneous canine gliomas, in order to correlate the angiogenic activity with their prognosis.

## MATERIAL AND METHODS

The study was performed with eighteen canine gliomas, including **12 anaplastic oligodendrogliomas (AODG)**, **3 oligodendrogliomas (ODG)** and **3 glioblastomas (GB)**. We have evaluated both histological and immunohistochemically the main microscopical and vascular features. The expression of both markers in capillaries was evaluated considering representative areas from the centre and periphery of the tumour (Fig. 2). With **glomeruloid capillaries**, **vascular mimicry** and **vascular co-option**, the proportion of area stained with the marker was estimated.

Table 1. Immunohistochemical markers used for the study of canine gliomas: main features.

	Antibody name	Company	Dilution
CD31	Mouse anti-human CD31 Monoclonal antibody	Dako Mo82	1:20
VEGFR- 2	Rabbit anti VEGFR receptor 2 polyclonal antibody	Ab2349	1:25

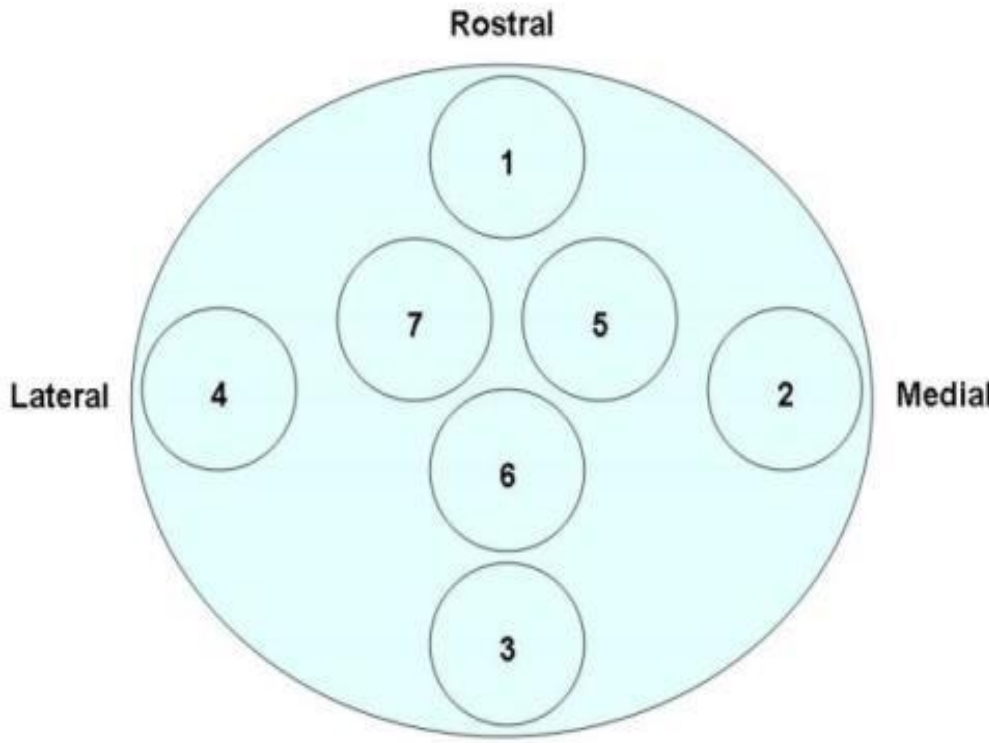


Fig. 2. Representation of the areas taken at 40x to count the capillaries.

RT, room temperature; WB, water bath; PC, pressure cooker.

## RESULTS

- High Grade Gliomas (HGGs) were the only ones that presented **Glomeruloid capillaries** and **vascular mimicry**, specially in GB. **Vascular co - option** was mainly detected in OGD and was not present in GB.

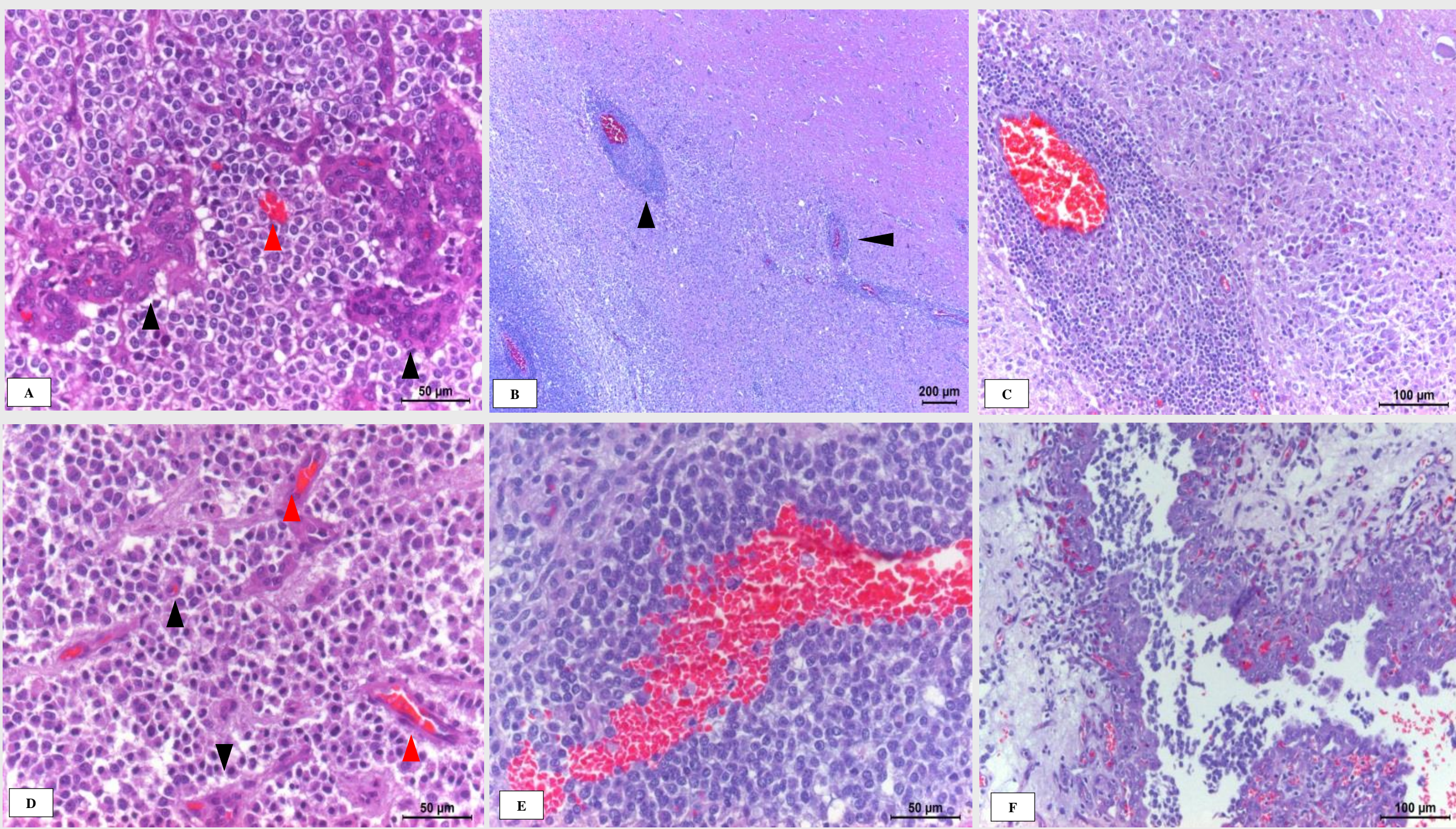


Fig. 3. Histopathological features of canine gliomas. (A) glomeruloid capillaries (black arrow) and hemorrhages (red arrow) in AODG. (B, C) Perivascular cuffs in different areas of the tumour (black arrow) with the cells orientated towards the vessel to get nutrients, typical of vascular co-option in AODG. (D) Transition between capillaries (red arrow) and glomeruloid capillaries (black arrow) can be seen in AODG. (E) Neoplastic cells orientated and surrounding red blood cells, looking like a vessel, typical of vascular mimicry in GB. (F) Glomeruloid capillaries in GB.

- Low Grade Gliomas (LGGs) presented higher positivity than HGGs for **CD31** and **VEGFR-2**. No expression of **VEGFR-2** and **CD31** in **vascular mimicry** and **vascular co - option** features was found in any tumour.

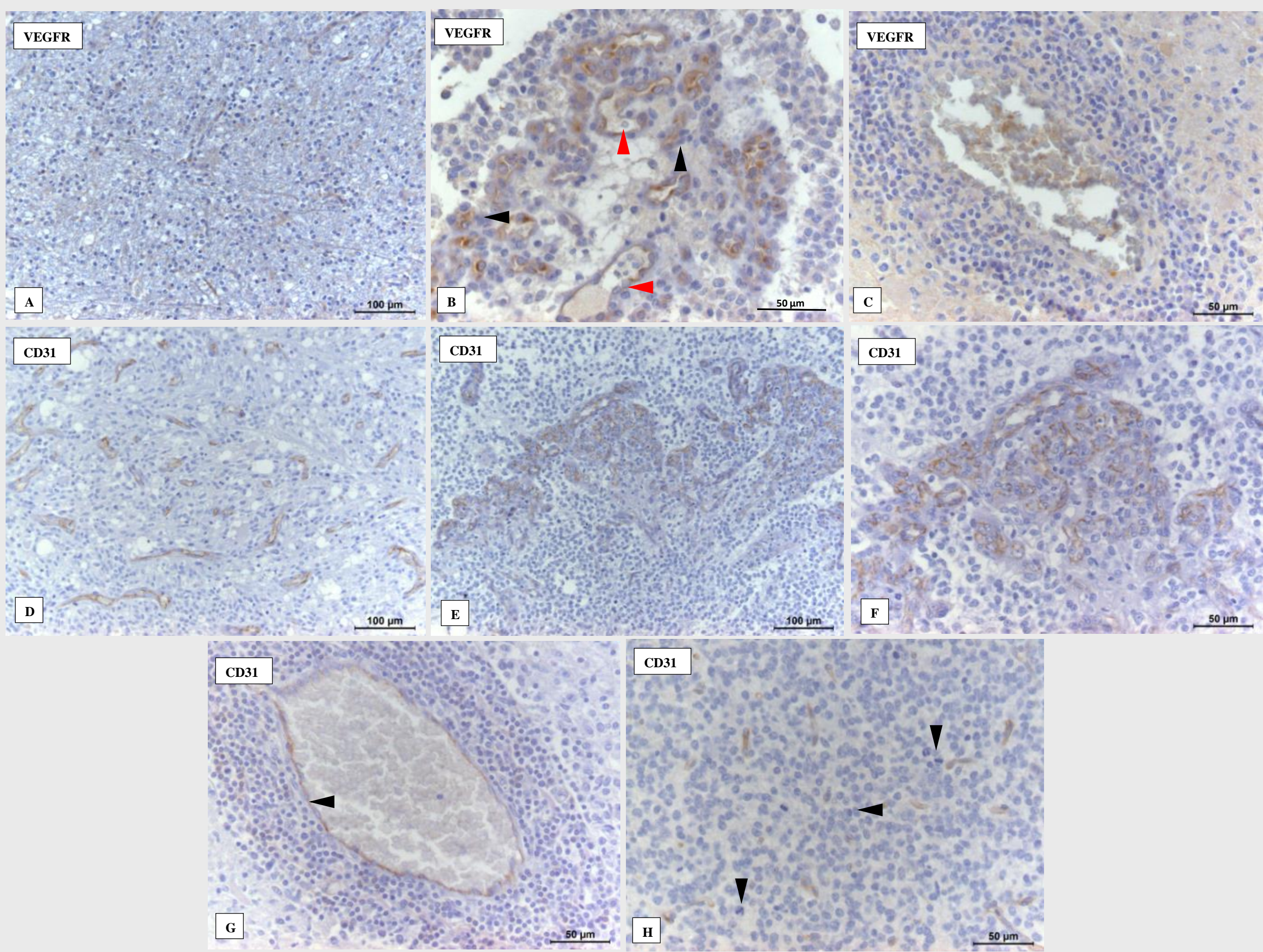


Fig. 4. (A) Medium proportion score Immunolabeling was observed in capillaries in AODG. (B) Glomeruloid capillaries showing low proportion score immunolabeling (black arrow). Capillaries (red arrow) showed a high proportion score in AODG. (C) vascular co-option in AODG. Neoplastic cells surrounding immunostained vessel are noticed. (D) Capillaries showing a high proportion score immunolabeling in AODG. (E, F) A high proportion score immunolabelling observed in glomeruloid capillaries in AODG (G) Vascular co-option in AODG. Note the neoplastic cells surrounding an immunostained vessel (arrow). (H) Mitosis (arrow) and immunostained capillaries in AODG.

## DISCUSSION

**Vascular features** found in canine gliomas showed a similar pattern of expression of their human counterparts<sup>1,4,5,6</sup>. LGGs showed Higher pattern of immunoreactivity for **CD31** and **VEGFR-2** in comparison with HGGs. There might be other mechanisms of neovascularization present in canine gliomas, independents of **VEGF**, like the glioblastoma endothelial cell transdifferentiation<sup>7</sup> that are increased in theses HGGs and could explain this lower immunostaining of endothelial cells in comparison with LGGs.

## CONCLUSION

With the markers used in this study was possible to identify angiogenic areas in canine gliomas, but further studies correlating their vascular features and the expression of these markers are needed to establish them as a prognostic factor.

## BIBLIOGRAPHY

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